Disentangling genes, attachment, and environment

Disentangling genes, attachment, and environment: A systematic review of the developmental psychopathology literature on gene–environment interactions and attachment

Lisa Golds MSc[1*], Karina de Kruiff MSc[1], Angus MacBeth PhD[1].

[1]University of Edinburgh, Scotland, UK.

Corresponding Author: Dr Angus MacBeth

School of Health in Social Science

The University of Edinburgh

Rm 3.06A

Doorway 6, Medical Quad

Teviot Place

Edinburgh, EH8 9AG

Tel: (+44) 650 3893

Fax: (+44) 651 3971

e-mail: angus.macbeth@ed.ac.uk

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Abstract

The role of genetics in relation to attachment is of continued interest to developmental psychology. Recent research has attempted to disentangle genetic main effects, environmental effects, and gene and environment (GxE) interactions in the development of attachment security /insecurity and disorganization. We systematically reviewed associations between gene markers and attachment, including GxE interactions, identifying 27 eligible studies. Inconsistent results emerged for associations between both gene effects and GxE interactions on attachment organization. Where GxE interactions used attachment as the environmental factor in the interaction, we observed more consistent results for differential susceptibility of GxE interactions on offspring behavior. Small sample size and heterogeneity in measurement of environmental factors impacted on comparability of studies. **From these results we propose that the future of research into the role of genetic effects in attachment, lies in further exploration of GxE interactions, particularly where attachment acts as an environmental factor impacting on other child developmental outcomes emerging from the caregiving environment, consistent with differential susceptibility approaches to developmental psychopathology. Importantly, from a methodological perspective, establishing the role of gene markers in such models will require a shift towards contemporary genomics, including** genome wide analysis (including novel genes and chromosomal loci), and epigenetic individual variations.

Keywords: Attachment, Genes, Gene x Environment, Disorganization

Introduction

Attachment has a pre-eminent position as a theory of child mental health and wellbeing, with implications for lifespan psychological development (Bowlby, 1969). One of the strengths of the theory is the interweaving of evolutionary, biological, and psychological constructs to give an integrated model of the development and maintenance of relational bonds between the child and the primary caregiver. **Therefore, an awareness of the relevance of underlying biomarkers in relation to attachment is long-standing**. Bowlby's formulation of attachment theory suggests that attachment to a primary caregiver provides the infant with a sense of security in the face of novel or stressful situations. Over time, repeated sensitive, congruent attachment interactions lead to the child's development of exploration (Letourneau, Giesbrecht, Bernier, & Joschko, 2014), resilience (Masten, 2001), emotion regulation (Denham *et al.,* 2003; Thompson, 1994), and the capacity to understand one's own and other's minds (Theory of Mind / mentalisation / mind-mindedness (Meins, Fernyhough, Wainwright, Das Gupta, & Fradley, 2002; Slade, 1999)) which in turn maximizes positive behavior and further relationships throughout the life course (Mikulincer & Shaver, 2007).

It has long been argued that sensitive, responsive parenting is vital in developing secure attachment within the child-caregiver dyad (Ainsworth, 1979; Chisholm, 1996). Attachment to sensitive caregivers confers a broad range of developmental benefits to children (Fraley, Roisman, Booth-Laforce, Owen, & Holland, 2013); including increased likelihood of secure classification on the Strange

Situation Test (Ainsworth, Blehar, Waters, & Wall, 1978), greater likelihood of developing positive peer relationships during early childhood (Kerns, 1994), as well as sustaining strong and trusting friendships into adolescence (Englund, Kuo, Puig, & Collins, 2011). Conversely, if children are exposed to insensitive, inconsistent, or abusive styles of parenting, then they are more likely to develop an insecure or disorganized style of attachment (Cyr, Euser, Bakermans-Kranenberg, & van IJzendoorn, 2010; Solomon & George, 1999). Children classified with disorganized attachment may often show ambivalence, anxiety or fear towards their caregivers and others, as well as displaying behaviors that are erratic and contradictory; leading to negative or misdirected externalized behaviors (Zeanah, Keyes, & Settles, 2003). In longitudinal studies, children classified with disorganized attachment as infants also display developmental problems in middle childhood, adolescence, and adulthood including aggressive behaviors and lower social competence (Solomon & George, 2011; Solomon, George & De Jong, 1995).

*The transmission gap and **modeling** of biomarkers for attachment*

Following from this, parental sensitivity was identified as a key mediator in the process by which attachment behaviors and representations are transmitted from parent to child, and how this impacts on child development, reflecting in the continuity (or perhaps the discontinuity) of patterns of attachment in the parent and those in the child. While parental sensitivity seems to be a critical factor, it has been suggested that its actual predictive power is inconsistent (De Wolff & van IJzendoorn, 1997), and evidence suggests that the correlation between parental and

offspring intergenerational association in attachment has dropped from r=.47 (van IJzendoorn, 1995) to r=.31 (Verhage *et al.,* 2016). **As robust meta-analytic data have shown, sensitivity explains less than 50% of the association between parent and infant attachment – summarized as the attachment "transmission gap" (van IJzendoorn, 1995; Verhage *et al.*, 2016). This has generated substantial research into identifying and modeling the effect of potential moderators on the relationship between parent and offspring attachment (van IJzendoorn & Bakermans-Kranenburg, 2019).** Whilst psychological environmental factors such as parenting styles, parental representations of attachment, and parental sensitivity undoubtedly play a role in developing attachment and in transmission of attachment patterns (Bakermans-Kranenburg & van IJzendoorn, 2007; Bernier, Matte-Gagne, Belanger, & Whipple, 2014), there has been considerable interest in modeling of biological markers for transmission of attachment, although work from a behavioral genetic perspective has failed to produce consistent markers for intergenerational transmission (e.g. Bokhorst *et al.,* 2003; Roisman & Fraley, 2008**). In this respect, the transmission gap has also acted as a driver towards investigating the role of genetic biomarkers in the attachment literature. In the current review, we will focus on the role of genetics in terms of associations with attachment and child outcomes, rather than in their contribution to the transmission gap per se.**

The traditional approach to modeling genetic and behavioral influences has generally focussed on one of two paths. First, the impact of the individuals own biological make-up on behavior can be considered in relation to the parent-child relationship. In their classic work, Thomas and Chess (1977) argued that from a very

young age, infants exhibit varying degrees of emotional temperament, which in turn may impact upon the behavior and the developing relationship between mother and child. This constitutes an example of biological makeup influencing the environment. Second, there may be instances where the environment directly impacts and influences an individual's biology. For example, unresponsive caregiving and an insecure attachment could lead to changes within the hypothalamic–pituitary–adrenal (HPA) system, leading to changes in stress response and emotion regulation (Hertzman & Boyce, 2010). However, **due to the multiple systems in which a child develops, these individual biological and environmental characteristics cannot be viewed as though they are working in isolation (Esposito, Setoh, Shinohara & Bornstein, 2017) and so in contemporary developmental research, a rapprochement has emerged around nature vs nurture, focusing on how these genetic x environmental (GxE) elements work together**, both impacting and being impacted upon to create unique phenotypes within each individual child (Letourneau *et al.,* 2014).

Gene x Environment (GxE) studies

Early ("first wave") GxE studies used a dual risk approach (Sameroff, 1983), whereby the gene acts as a filter, with the environment passing through it, and the filter straining out negative factors (Letourneau *et al.,* 2014). In this way, if the environment is optimal then the filter has no job to do, but an imperfect filter would be of little use in poor conditions **and could cause**, in the case of the child, developmental difficulties later on. Much of the early findings on genetic heritability of attachment take this approach, focusing on a twin study methodology (critically

reviewed in Barbaro, Boutwell, Barnes, & Shackelford, 2017). Furthermore, the behavioral genetic approach also delineates between shared and non-shared environments, with the majority of attachment-based twin research measuring attachment via the shared environment acting upon the twins (e.g. parental caregiving), without taking into account the longitudinal impact of non-shared environments e.g. individual life events such as trauma. Furthermore, as the attachment relationship emerges as the result of interactions between the caregiver and child in the first year of life, attachment organization can itself be viewed as a proxy for the environment, introducing additional complexity into the modeling of the transmission of attachment organization.

Contemporary research on GxE as related to attachment adopts a more nuanced position that some genes may act with greater or lesser plasticity; and correspondingly the gene may cause atypical development in poor conditions, but may enhance positive development in an encouraging environment or vice versa (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011). In these "differential susceptibility" models, the gene may be responding more uniquely to the environment in which it is found. As Bakermans-Kranenburg and van IJzendoorn (2006) suggest, the most important effects of biology on attachment may be moderated by the environment in which the child finds him or herself. For example, children living in institutionalized care, who were identified as carrying a specific gene variation, showed a higher likelihood of attachment disorganization than children with the same gene variation who were raised in foster homes (Bakermans-Kranenburg, Dobrova-Krol, & van IJzendoorn, 2011).

Over the last two decades, a number of longitudinal cohorts have reported on the interactions between candidate genes and environmental factors (e.g. caregiving setting, parental sensitivity, and so on). Within these research cohorts, evidence has accumulated to support the view that specific genes do interact with the environment to influence attachment, and therefore secure attachment and attachment disorganization **may** be predicted by the presence or absence of specific candidate genes. However, there is little consensus as to which genes have a significant impact, and as with many approaches to GxE in developmental psychopathology there are difficulties in replicating significant findings (Papageorgiou & Ronald, 2017). Additionally, many studies are hampered by small sample sizes; making it difficult to state a definitive association between gene-environment interactions and their impact on attachment (Hygen, Guzey, Belsky, Berg-Neilson, & Wichstrøm, 2014; Luijk, Roisman *et al.,* 2011). Furthermore, in these approaches the effects of GxE interactions on attachment concern patterns of association between genes, specific environmental factors, and their impact on attachment organization as an outcome. Alternatively, it is also possible to delineate GxE interactions involving attachment whereby the gene marker interacts with attachment (as the "E" marker) to impact on a given developmental outcome (e.g. problem behavior (Li et al., 2016).

Potential candidate genes identified within attachment studies

Most of the research surrounding this area of study have concentrated on a small number of candidate genes that have been proposed as influential upon

attachment organization. These studies can be considered to represent genes as main effects on attachment as an outcome. The candidate gene association approach assumes an association between measured characteristic and gene, enabling identification of variance in the association. From a developmental psychopathology perspective, likely candidate genes can be identified among the dopamine, serotonin, and oxytocin systems. These neurotransmitter systems are intimately connected to the development and operation of affect processing and emotion as experienced by the child, as well as being implicated in the formation of social bonds between humans (Luijk, Roisman, *et al.,* 2011).

Notable candidate genes within the dopamine system include DRD4, DRD2, and COMT variants. Firstly, with regard to dopaminergic systems, the dopamine D4 receptor (DRD4) is a significant genetic marker for cognitive and emotional processes in the prefrontal cortex (PFC) (Wang *et al.,* 2004). As part of the dopaminergic system, it is also argued that DRD4 is related to concentration and attention levels and that this too may affect the attachment bond that develops between a child and primary caregiver (Graffi *et al.,* 2015). Research suggests that carriers of the DRD4 7-repeat allele show lower levels of dopamine reception (Bakermans-Kranenburg & van IJzendoorn, 2011). With this in mind, a link between DRD4 genotyping and attachment disorganization could be reflective of alterations in the function of these attachment-related cognitive systems. Similarly, the A1 allele of the DRD2 gene has been linked to a reduced binding effect of dopamine, leading to lower levels of dopamine in the system (Jönsson *et al.,* 1999).

Secondly, the gene coding for COMT, an enzyme which works to break down the dopamine, epinephrine, and norepinephrine in the PFC is dependent upon the homozygous or heterozygous Val/Met allele. COMT is responsible for more than 60% of the dopamine breakdown in the PFC (Li *et al.,* 2016), and individuals carrying the Val/Val genotype show COMT activity increased by fourfold compared to those carrying a Met/Met genotype (Hygen *et al.,* 2014). This suggests that children carrying the Val/Val genotype will have lower levels of dopamine within their system, which may ultimately impact on the ways in which they interact with primary caregivers.

Thirdly, within the serotonin system, 5HTTLPR acts to impact upon stress levels and anxiety (Leerkes *et al.,* 2017; Zimmerman, Mohr, & Spangler, 2009). The short (s) allele of 5HTTLPR has been connected to lower efficiency compared to the long (l) allele, (Lesch *et al.,* 1996), which in turn suggests that individuals who are carriers of the (s) allele (s/s or s/l) could be more susceptible to anxiety and stress than homozygous carriers of the (l) allele (l/l) (Sen, Burmeister, & Ghosh, 2004). This has potential implications for attachment systems, as infants exhibiting higher levels of stress and anxiety may experience greater difficulty in forming trusting bonds with primary caregivers.

Finally, OXTR has been highlighted as a candidate gene within the oxytocin system. As the oxytocin system is related to human empathy and bonding (Carter, 1998), there are clear parallels to the social-affective interaction behaviors seen in attachment care-giving and receipt. It has been suggested that carriers of the GG allele of OXTR have higher levels of social cognition leading to increased prosocial

behavior (Bartz, Zaki, Bolger, & Ochsner, 2011). This could impact on attachment security, as children with this gene may be more prone to exhibit behaviors that appeal to parents, from an early age.

In addition, as further research is undertaken within the field, other novel genes are being identified as possible candidate biomarkers. These genes go beyond what have been called the "usual suspects" (Ebstein, Israel, Chew, Zhong, & Knafo, 2010; Pappa *et al.,* 2015), identifying additional biological systems that are influencing and influenced by the environment in which the child develops. Specific genes, and their pathways, such as HDAC1, ZNF675 and BSCD1 have been linked to disorganized attachment (Pappa *et al.,* 2015), and FKBP5 and related single nucleotide polymorphisms (SNPs) are recognized as focal due to their connections to the glucocorticoid receptor (GR) and mineralocorticoid (MR) systems which interact with the HPA-axis during stressful experiences (Ising *et al.,* 2008). **More recently, within the GR system, NR3C1 methylation has also been identified as a possible mediator of attachment between parent and child, when external environmental factors are taken into consideration (Bosmans, Young & Hankin, 2018).** This extension of research into molecular genetics, and the tentative links that are made to environmental interaction may represent future avenues for exploration of GxE interactions. There is also the additional question of interactions between gene markers on attachment outcomes (gene x gene "GxG" effects; see Popper *et al.,* 2006; Cicchetti *et al.,* 2011).

However, given the aforementioned inconsistencies in the literature, there are limits to the confidence with which we can state that there are meaningful GxE

associations in attachment. In addition, there is a need to more clearly delineate distinctions between attachment as an environmental factor in a GxE interaction on a child outcome, and attachment as a child outcome variable influence by a GxE interaction. Although there have been a number of narrative overviews of the genetics of attachment (Bakermans-Kranenburg & van IJzendoorn, 2007), to date it seems that there has been no systematic review and synthesis of the existing literature on GxE interactions impacting on attachment. Furthermore, many of the authors of previous studies have reported contradictory findings to that of their peers, and have themselves argued that inconsistent findings offer little to predict the conditions in which candidate genes affect attachment directly, or interact with the environment to impact attachment (Leerkes *et al.*, 2017; Roisman, Booth-Laforce, Belsky, Burt, & Groh, 2013).

Given the rapidly accumulating evidence around the genetics of attachment we therefore aimed to systematically collate, synthesize, and critically evaluate the data that has thus far been presented within the area of GxE interactions and attachment. This literature incorporates multiple cohort studies and intervention trials.

The primary aims of the review were to examine the strength of association between candidate genes and i) child attachment security/insecurity; and ii) child attachment disorganization. The review sought to ascertain whether reported associations were significant, and if any consistent patterns of association could be established between attachment organization and specific gene markers. A second aim was to identify the extent to which external environmental influences may

impact on attachment outcomes via GxE associations, and thirdly, to assess whether the existing literature identifies associations between candidate genes and attachment classification (where attachment forms the "E" in a GxE interaction) upon child developmental outcomes. Finally, the review appraised methodological sources of bias in the current literature.

Methods

Inclusion and Exclusion criteria

A systematic search was conducted using PRISMA guidelines (Moher, Liberati, Tetzlaff & Altman, 2009). The inclusion criteria identified articles that (i) reported on original primary data; (ii) measured attachment of the child to a primary caregiver; (iii) included a population sample of children aged 18 years old or younger; (iv) identified specific genotyping; (v) presented statistical data on any association, or lack thereof, between specific genetic markers and attachment of the child-carer dyad, or external environmental factors; (vi) were published between 1990-2017; and (vii) were written in the English language. During extraction, it was also noted whether the studies had identified any significant environmental factors that may have impacted upon the associations that they reported. This allowed for identification of studies that concentrated purely on genetic influence or for GxE impact on attachment. In order to eliminate overlap of data when using cohort studies with the same population sample, separate papers were only included if it was found that they reported on different genetic markers, different haplotypes or SNPs of genetic markers, or different external environmental influences

Exclusion criteria comprised (i) articles that discussed associations between genetic markers and attachment but presented no statistical data; (ii) book chapters summarizing findings of previous studies; (iii) previous systematic reviews, which again only summarized previous findings; and (iv) reports using non-human samples. Twin studies were excluded from the review, as these concerned behavioral genetics, rather than specific genetic biomarkers.

Literature Search

Relevant studies were initially identified via an electronic database search of OVID (comprising PsycINFO 1806 to November Week 1 2018, Embase Classic+Embase 1947 to 2018 November 1, MEDLINE®, Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid), Applied Social Sciences Index and Abstracts (ASSIA), and Google Scholar. The search terms were developed in consultation with a specialist librarian and were as follows:

attachment AND (*behavio** OR *organi** OR *disorgani**) AND "*DRD-4*" OR *drd4* OR *COMT* OR "*Val/Met*" OR "*Val/Val*" OR "*5-HTTLPR*" OR *5httlpr* OR "*g x e*" OR "*gene x environment*".

The year of publication was limited from 1990–2018, as this was deemed to be a period long enough for capturing the advanced molecular genetic results necessary, and language was limited to English. Truncation [*] was employed to increase the sensitivity of the search to include both American English and British English

spelling, as well as to allow a number of word-ending inflections that would broaden the literature search (eg. disorganized, disorganization etc..). Duplicates across the various databases were then removed, and inclusion and exclusion criteria were applied to titles, abstracts, and full texts. The search strategy and identification of eligible studies was independently conducted by two researchers.

After confirming studies which met inclusion and exclusion criteria at full text level, reference lists of all included papers were checked to ascertain that no additional studies of relevance had been overlooked during the primary search. These additional studies were then also subject to a thorough scrutiny using the inclusion and exclusion criteria at abstract and full text level. Disagreements over inclusion between the two researchers was resolved through consensus discussion with a third researcher not involved in the initial search process. For details of the search process see Fig. 1.

Outcomes

Outcomes were characterized as reporting a significant association between genetic markers and attachment classification, or a significant two-way association between genetic markers, environmental impact, and attachment classification. For the purposes of clarity, *environmental impact* was defined as any influence upon the child that was not caused by any genetic effect (Beaver, Eagle Shutt, Vaughn, DeLisi, & Wright, 2012). A significant association was defined as having a *P* value of 0.05 or less. Any significant associations were then reported to compare and contrast the published results (see Tables 2 and Table 3 for further details).

Quality Assessment

The risk of quality assessment bias for all included studies was carried out using an adapted version of the 'Agency for Healthcare Research and Quality' (AHRQ) checklist (Williams, Plassman, Burke, Holsinger, & Benjamin, 2010). For this review, the AHRQ was specifically adapted to test each paper against 11 criteria to ensure that author bias had been minimalized, and that limitations for each study had been addressed openly. The 11 criteria comprised (i) unbiased selection of cohort; (ii) selection minimizes baseline differences; (iii) sample size calculated; (iv) adequate description of cohort; (v) validated method for ascertaining attachment status; (vi) validated method for ascertaining participant genotype; (vii) outcome assessment blind to exposure; (viii) adequate follow-up period (longitudinal studies); (ix) missing data/drop out addressed; (x) analysis controls for confounding variables; and (xi) analytic methods appropriate. The outcome of each criteria was then entered into a scoring system and could be assigned a number of ratings including; Yes = (2), Partially = (1), No = (0), and N/A = (0), allowing scores to range from 0 - 22. The AHQR was conducted by two researchers and scores were compared. On an individual item level, inter-rater agreement ranged from 84 - 100%, with Kappa values ranging from 0.6 – 1. For the final total scores for each study, the inter-rater agreement score was 68% with a Kappa value of 0.6 indicating substantial agreement. After full analysis, any studies which showed discrepancies between scores were reassessed.

Results

Characteristics of the studies

In total, 27 studies were included in the review. All of the studies used primary data and **24 used a prospective cohort design** (Bakermans-Kranenburg *et al.,* 2011; Barry, Kochanska, & Philibert, 2008; **Borelli, Smiley, Rasmussen, Gómez, Seaman, & Nurmi, 2017; Bosmans *et al.*, 2018;** Cicchetti, Rogosh, & Toth, 2011; Gervai *et al.,* 2005; Graffi, 2016; Graffi *et al.,* 2015; Humphreys, Zeanah, Nelson, Fox, & Drury, 2015; Hygen *et al.,* 2014; Kochanska, Philibert, & Barry, 2009; Lakatos *et al.,* 2000; Lakatos *et al.,* 2002; Leerkes *et al.,* 2017; Li *et al.,* 2016; Luijk *et al.,* 2010; Luijk, Tharner *et al.,* 2011; Pappa *et al.,* 2015; Propper, 2006; Raby *et al.* 2012; Spangler, Johann, Ronai, & Zimmerman, 2009; van IJzendoorn & Bakermans-Kranenburg, 2006; Viddal, Berg-Neilson, Belsky, & Wichstrøm, 2017; Zimmerman *et al.,* 2009)

Within **the 24 studies** that employed a prospective cohort design, **16 cohort samples were identified**. A number of cohorts were used across multiple studies; where the sample population was used to understand a variety of genetic or environmental influences. The Generation R cohort (The Netherlands) was used across 3 studies; Luijk *et al.* (2010), Luijk, Tharner *et al.* (2011), and Pappa *et al.* (2015), using data collected between 2003-2005. The Maternal Adversity, Vulnerability and Neurodevelopment Project (MAVAN) (Canada) was sampled for 2 studies; Graffi (2016) and Graffi *et al.* (2015) with data collected between 2003-2009. Spangler *et al.* (2009) and Zimmerman *et al.* (2009) both used data collected from the Regensburg Longitudinal Study (Germany), with longitudinal data collected between 1974-2005. 2 studies used a sample population from the Trondheim Early Secure Study (TESS)

(Norway); Hygen *et al.* (2014) and Viddal *et al.* (2017), using data collected between 2007-2011. The Budapest Infant Parent Study (BIPS) (Hungary) was used across 3 studies; Gervai *et al.* (2005), Lakatos *et al.* (2000), and Lakatos *et al.* (2002). 2 studies were also published by the University of Iowa; Barry *et al.* (2008) and Kochanska *et al.* (2009). The remaining studies were published by individual cohorts; most originating from universities or health centres. The samples were researched in a number of countries including The Netherlands (van IJzendoorn & Bakermans-Kranenburg, 2006), USA (**Borelli *et al.*, 2017; Bosmans *et al.*, 2018;** Cicchetti *et al.,* 2011; Leerkes *et al.,* 2017; Li *et al.,* 2016; Propper, 2006; Raby *et al.,* 2012), Ukraine (Bakermans-Kranenburg *et al.,* 2011), and Romania (Humphreys *et al.,* 2015).

The 3 remaining studies; Brumariu, Bureau, Nemoda, Sasvari-Szekely, and Lyons-Ruth (2016), Gervai *et al.* (2007), and Luijk, Roisman *et al.* (2011) employed cross-cohort designs. Luijk, Roisman *et al.* (2011) compared findings between the Generation R study (The Netherlands) and the Study of Early Child Care and Youth Development (SECCYD) (USA). The papers by Brumariu *et al.* (2016), and Gervai *et al.* (2007) reported findings from combined cohorts drawn from the Budapest Infant Parent Study (BIPS) (Hungary) and Harvard Medical (USA). Characteristics of all studies are described in Table 1.

Sample population

The total population for the included studies was **n=6347 infants and children, representing 18 cohorts**, (**27 studies including multi-cohort samples**); with individual samples ranging from n=37 (Bakermans-Kranenburg *et al.,* 2011) to

n=1854 (Luijk, Roisman *et al.,* 2011). Based on the studies that reported gender (k=21; where k=number of studies), 51.1% of the participants were male and 48.9% of the participants were female. The children within the studies ranged in age from 3 months **– 16 years old**. Sixteen of the studies across the review observed the GxE impact on attachment in infants aged between 3 – 18 months. Nine of the 25 studies observed children older than 18 months; ranging from 18 months to 8 years old, **and three of these studies** examined children into adolescence **up to 16 years old.**

Measurement of attachment

Of the studies which measured attachment classification in infants (n=18), all used the Strange Situation Procedure (SSP) to measure attachment between child and primary caregiver. As described by Ainsworth *et al.* (1978), the SSP consists of mildly stressful events including separation from caregiver and the introduction of a stranger, ending in reunion with the caregiver. Attachment was measured in all cases when the infant was between 12-18 months old. In one study of children aged between 3 - 6 years (Bakermans-Kranenburg *et al.,* 2011) the SSP was also employed. This was also the case in one study of children aged approximately 42 months (Humpreys *et al.,* 2015). As some of the children within the Humphreys *et al.* (2015) study were raised in institutionalized care, attachment was measured between the child and the caregiver with which they had spent the most time, and appeared to be most attached to. It is not noted whether either of these studies used a modified version of the SSP for children older than 24 months.

A modified version of the SSP was utilized in 2 studies administered to

children aged approximately 36 months (Graffi, 2016; Graffi *et al.,* 2015). The modified SSP as described by Cassidy and Marvin (1992) consists of four episodes of separation and reunion and is recommended for use with children of preschool age. Two included studies focusing on middle childhood (Hygen *et al.,* 2014; Viddal *et al.,* 2017) used the Manchester Child Attachment Story Task (MCAST). The children within these studies were between the ages of 4 and 6 years old at the time of testing. The MCAST, as described by Green, Stanley, Smith and Goldwyn (2000), incorporates age-appropriate aspects of both the SSP and the Adult Attachment Interview (AAI), ensuring that the child is able to convey their feelings through a simple narrative. **In the studies sampling adolescents, a number of resources were used. Borelli *et al.* (2017) used the Security Scale (SS); a 15-item questionnaire that is used to measure self-esteem (Borelli *et al.*, 2017). Bosmans *et al.* (2018) measured anxious and avoidant attachment with the Experiences of Close Relationships-Relationship Structure Questionnaire (ECR-RS). This is a 10-item self-report questionnaire designed to measure anxious and avoidant attachment styles (Fraley, Heffernan, Vicary, & Brumbaugh (2011).** Zimmerman *et al.* (2009) used the Late Childhood Attachment Interview (LCAI) in their follow up of children within the Regensburg Longitudinal Study at 12 years old. The LCAI comprises a semi-structured interview in which the child has the opportunity to discuss their feelings of attachment to their caregivers, which can then be attributed to attachment representations (Zimmerman *et al.,* 2009)

Attachment was categorized using a number of validated approaches. A continuous measure of attachment security was employed by 6 studies (Bakermans-Kranenburg *et al.*, 2011; Humphreys *et al.*, 2015; Leerkes *et al.*, 2017; Luijk, Tharner *et*

al., 2011; Pappa *et al.*, 2015; and Viddal *et al.*, 2017). Zimmerman *et al.* (2009) used the "attachment behaviour strategy scale" in line with the LCAI designed for adolescents. The remainder of the studies employed the traditional attachment classification categories (A, B, C, D).

Sampling of Genetic markers

All of the genes across the studies were collected from infants and children using validated methods **including saliva samples (k=4),** buccal cheek/mouth swabs (k=19), and cord blood samples (k=4). Cord blood sampling is known to be associated with contamination from maternal genetic material (Morin *et al.* 2017). However, sensitivity checks of this sampling indicated that contamination was present in less that 1% of cases; and where contamination was present data were excluded from further analyses (Luijk *et al.,* 2010). Gervai *et al.* (2005) report collecting genetic information from both the child and the parent, in an attempt to understand generational transmission rates.

A number of genetic markers were identified within studies using a candidate gene approach. Some studies concentrated on one specific gene whereas other studies broadened their approach and incorporated more than one genotype into their testing. The two main genes examined were located in dopaminergic and serotonergic systems, which were each investigated in 14 studies. Of the "usual suspects" dopaminergic and serotonergic candidate genes, 5HTTLPR was tested for in all 14 studies (Bakermans-Kranenburg *et al.*, 2011; Barry *et al.,* 2008; Brumariu *et al.,* 2016; Cicchetti *et al.,* 2011; Gervai *et al.,* 2007; Humphreys *et al.,* 2015; Kochanska

et al., 2009; Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011; Proppa, 2006; Raby *et al.,* 2012; Spangler *et al.,* 2009; Viddal *et al.,* 2017; Zimmerman *et al.,* 2009); DRD4 was tested for in 12 studies (Cicchetti *et al.,* 2011; Gervai *et al*., 2005; Gervai *et al.,* 2007; Graffi, 2016; Graffi *et al.,* 2015; Lakatos *et al.,* 2000; Lakatos *et al.,* 2002; Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011; Proppa, 2006; Spangler *et al.,* 2009; van IJzendoorn & Bakermans-Kranenburg, 2006), seven of which also investigated the effect of the -521 C/T SNP promoter (Cicchetti *et al*., 2011; Gervai *et al.,* 2005; Lakatos *et al.,* 2002; Propper, 2006; Spangler *et al.,* 2009; van IJzendoorn & Bakermans-Kranenburg, 2006); COMT in 4 studies (Hygen *et al.,* 2014; Leerkes *et al.,* 2017; Li *et al.,* 2016; Luijk, Roisman *et al.,* 2011); and DRD2 in 3 studies (Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011; Proppa, 2006). In addition, OXTR was investigated in 2 studies (Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011). Further details of the studies, genetic alleles, SNPs, and haplotypes that were identified with each gene are recorded in Table 1.

With regard to de novo candidate genes, polymorphisms BclI rs41423247, TthIIII rs10052957, GR-9b rs6198, N363S rs6195, ER22/23EK rs6189 and rs6190, within the GR receptor gene were discussed in 2 studies (Luijk *et al.,* 2010; Luijk, Tharner *et al.,* 2011), and the MR receptor gene was discussed in 1 study (Luijk, Tharner *et al.,* 2011). Additionally, genetic markers known as **FKBP5 (Borelli *et al.,* 2017; Luijk *et al.,* 2010), NR3C1 (Bosmans *et al.,* 2018),** as well as HDAC1, ZNF675, BSCD1, and CACNAZD3 (Pappa *et al.,* 2015) were all identified as genes of interest **across one or more of the studies**. Finally, in the only study of its type included in the review Pappa *et al.* (2015) performed a Genome Wide Association Study

identifying suggestive loci on chromosomes 3p21, chr12q24, chr5q15, chr3q23, chr7q11, chr2q31, chr3p25, and chr6q12.

Environmental factors identified

A number of candidate environmental factors were identified across the studies and measured for their associations with attachment organization. These factors are considered additional to identifying attachment in itself as an environmental factor. These included parental mental health (k=2), parenting style (k=13), physiological responses of the child (k=2), physical attributes (k=1), and living situation (k=3). Furthermore, given the established importance of maternal sensitivity in attachment organization, this was measured **in 12 studies**. A further **7 studies (Borelli *et al.*, 2017;** Humphreys *et al.,* 2015; Hygen *et al.,* 2014; Kochanska *et al.,* 2009; Li *et al.,* 2016; Viddal *et al.,* 2017; Zimmerman *et al.,* 2009) identified the attachment status between child and caregiver as the candidate environmental factor; observing the interplay between genetic marker and attachment to moderate externalized behaviors. Three studies (Gervai *et al.,* 2005; Lakatos *et al.,* 2000; Lakatos *et al.,* 2002) included within the review did not identify environmental factors, reporting only on candidate gene associations with attachment. Additional covariates identified in each study are summarized in Table 1.

Significant associations between gene and attachment classification

As the studies within the review observed a number of different genetic markers, and to aid the delineation of patterns and trends, the results are presented

subdivided across the candidate genes. Full details of findings, significance, and covariates are displayed in Tables 2 and 3.

Serotonin markers: 5HTTLPR

With regard to associations between candidate genes and attachment, only 4 of 14 studies found any significant associations between 5HTTLPR and attachment organization. Propper (2006) reported a significant association between 5HTTLPR s/s alleles and lower avoidant attachment behaviors during the SSP, specifically during episode 8 (reunion with caregiver). In addition, Barry *et al.* (2008) reported an association between 5HTTLPR and attachment security, and Luijk, Roisman *et al.* (2011) also reported that there is a significant association between the 5HTTLPR short allele (s/s or s/l) and increased attachment security although this was only observed in one of the population samples (Generation R) and was not correlated in their second sample (SECCYD). Conversely, Spangler *et al.* (2009) reported a significant association between the 5HTTLPR short allele and attachment disorganization, but not attachment security.

Dopamine markers: DRD4, DRD2, COMT

DRD4 was reported to have shown a significant association with attachment status in 5 out of 12 studies. However, as with 5HTTLPR the results observed across the studies were inconsistent. Lakatos *et al.* (2000) reported that the DRD4 7-repeat allele was found more often in infants who displayed disorganized attachment. In a later study, albeit from the same cohort sample, Lakatos *et al.* (2002) also added to

their findings that the presence of the -521 T allele paired with the DRD4 gene significantly increased the risk for infants of expressing a disorganized attachment with their primary caregiver. In addition, Gervai *et al.* (2005) reported that there was a higher than expected transmission of the DRD4 7-repeat allele from parents to infants who also exhibited a disorganized attachment. Graffi *et al.* (2015) reported a significant association between children without the DRD4 7-repeat allele and disorganized attachment. A later study by Graffi (2016) reiterated the findings that children without the DRD4 7-repeat allele were more likely to exhibit disorganized attachment than children with the DRD4 7-repeat allele. A genetic influence was also observed by Propper (2006) which showed a significant association between DRD2 A1/A2 polymorphisms and lower avoidant attachment scores during episode 8 of the SSP (reunion with caregiver).

Finally, while COMT was tested for in 4 studies, only 1 study reported a significant correlation between the gene and disorganized attachment. Luijk, Roisman *et al*. (2011) examined the COMT gene in relation to disorganized attachment, reporting a significant correlation in both the Generation R and SECCYD cohorts.

Oxytocin markers: OXTR

A significant association was reported between OXTR heterozygotes and classification of infant disorganized attachment by Leerkes *et al.* (2017) in their full sample. However, when these analyses were delineated by racial subgroups (African American or European American) no significant patterns of association were

identified. In the second study concentrating on OXTR (Luijk, Roisman, *et al.,* 2011) no associations were found.

Additional genetic markers

As noted above, an emerging strand of work has identified potential de novo genetic markers. For instance, a number of the novel genetic markers reported by Pappa *et al.* (2015) reported significant associations with attachment status. Presence of the BECN1 gene predicted a significant association for attachment security. The HDAC1, ZNF675, and BSCD1 genes, in conjunction with synaptic transmission pathways and cation transport, showed a significant association with disorganized attachment. Within this study five suggestive loci on various chromosomes; 3p21, chr12q24, chr5q15, chr3q23, and chr7q11, also suggested significant correlations to disorganization. However, these marker findings are as yet unreplicated.

Significant two-way associations between gene, environment and attachment classification

Alongside the literature on direct associations between genetic markers and attachment (main effects of genes on attachment) there is also a substantial body of literature incorporating consideration of environmental factors as outlined above. Several studies investigated the impact of environmental factors as moderators or mediators of the reported associations between genetic markers and attachment – both with regard to attachment security (Table 2) and disorganization (Table 3). We consider these studies as GxE studies with an attachment outcome.

Serotonin markers: 5HTTLPR

Parenting styles were measured as an environmental factor in many of the studies, and 5 of 8 studies reported a significant association between 5HTTLPR, parenting, and attachment status. Firstly, Propper (2006) reported that children with the 5HTTLPR long (l/l) allele exhibited a greater degree of avoidant attachment behaviors, when exposed to negative parenting compared to children carrying the short (s/s or s/l) allele. Secondly, Barry *et al.* (2008) reported that children carrying the 5HTTLPR short (s/s or s/l) allele, when exposed to high levels of parental responsiveness, exhibited a greater degree of attachment security compared to children with the 5HTTLPR long (l/l) allele. In contrast, Cicchetti *et al.* (2011) reported that non-maltreated children with the 5HTTLPR long (l/l) allele showed higher levels of secure attachment, however this was not replicated in children who were maltreated at home.

In addition, there was also some evidence for an association between 5HTTLPR and disorganized attachment status. Spangler *et al.* (2009) reported that children with 5HTTLPR were more likely to have disorganized attachment when exposed to poor maternal responsiveness, and Bakermans-Kranenburg *et al.* (2011) reported that children with the 5HTTLPR short allele were significantly more at risk of developing disorganized attachment when placed in an institutionalized home, when compared to those in a family home.

Dopamine markers: DRD4, DRD2, COMT

With regards to dopamine markers, DRD4 was reported on in 5 studies in interaction with maternal sensitivity and parenting style. However as noted with regard to direct associations between dopamine and attachment results across studies are somewhat contradictory.

In the first instance, van IJzendoorn and Bakermans-Kranenburg (2006) reported a significant association between the DRD4 7-repeat allele and increased ratings of disorganized attachment, in the presence of the child being exposed to maternal unresolved loss / trauma. Findings by Gervai *et al.* (2007) also support this position, replicating a significant association between DRD4 and disorganized attachment, in this case in the context of the child's exposure to maternal disrupted communication. In addition, Gervai *et al.* (2007) also reported an association between the DRD4 short form (without the 7-repeat allele) and increased rates of disorganized attachment, when exposed to disrupted communication between child and mother. Further to this, Luijk, Roisman *et al.* (2011) reported a significant association between the absence of the DRD4 7-repeat allele and increased attachment security when the child experienced high levels of parental sensitivity. However, these results were only replicated within one half of their composite study (the SECCYD sample), whilst the opposite trend was reported within the Generation R sample. Similar to the Generation R cohort (Luijk, Roisman *et al.* 2011), significant associations were found between DRD4 and increased attachment security in the context of additional environmental factors. Firstly, associations between absence of the DRD4 risk allele and greater attachment security were observed in the context of positive maternal sensitivity (Leerkes *et al.* (2017). Secondly, there was an association

between DRD4 risk genotypes on classification as disorganized with respect to attachment at age 2, for children classified as non-maltreated (Cicchetti *et al.* 2011). The latter study also reported that an absence of DRD4 in maltreated children would lead to associations with disorganized attachment.

In contrast, in 2 studies (Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011) DRD2 showed no significant associations when interacting with environmental factors, and no findings were reported of an effect on attachment status. Finally, an interaction between COMT homozygosity and high parental sensitivity on reduced attachment disorganization was reported by Luijk, Roisman *et al.* (2011) within their Generation R sample. However, these results were not significantly replicated in the second half of the composite sample (SECCYD).

Oxytocin marker: OXTR

Leerkes *et al.* (2017) found a significant association between OXTR and attachment security, among African-American infants, when they were exposed to positive maternal sensitivity. Conversely, Luijk, Roisman *et al.* (2017) reported no significant associations.

Additional genetic markers

With regard to the possibility of novel genes interacting with maternal sensitivity and responsiveness, Luijk, Tharner *et al.* (2011) reported that children with the minor MR allele (G) within the HPA-axis developed increased attachment security, whereas those children who were exposed to maternal insensitivity and

unresponsiveness were more likely to have reduced attachment security**. Bosmans *et al.* (2018) reported that in children with anxious attachment, the interaction of NRC31 methylation and low maternal support could predict higher anxious attachment in the context of higher stress levels.** However, as with de novo findings for candidate gene associations, these novel GxE interactions are still subject to replication.

Gene x Gene interaction

Two studies addressed additive risks from gene x gene interactions. Propper (2006) reported that a gene x gene interaction of the 5-HTTLPR risk alleles and the DRD2 gene allele was associated with increased resistant attachment behavior on the SSP. Secondly, Cicchetti *et al.* (2011) also identified a significant association between combined risk genotyping of DRD4 and 5HTTLPR alleles and disorganized attachment at age 2 in children classified as non-maltreated. However, this finding was not replicated for children who were classified as maltreated, indicative of an additional environmental interaction.

Studies with no significant associations between genes, attachment and other environmental factors

In addition, 3 of the studies included within the review reported no significant associations between genetic markers or GxE influences on attachment. In studying the novel gene FKPB5 (Luijk *et al.,* 2010), no associations were reported between the genetic marker and an influence on attachment status. Raby *et al.* (2012) reported no associations between 5HTTLPR, or 5HTTLPR and maternal

responsiveness on attachment. Likewise, Brumariu *et al.* (2016) reported no associations between 5HTTLPR, or 5HTTLPR interacting with maternal behavior, and attachment. However, they did note that 5HTTLPR was significantly associated with the infant's proneness to distress.

GxE interactions of attachment on child outcomes.

In addition to studies focusing on GxE interactions on attachment outcomes, **7 studies** within the included corpus of studies reported on GxE interactions on outcome where the 'E' marker was identified as the attachment classification between the child and caregiver, and the outcome was another child developmental factor. Going beyond the testing of associations within the molecular GxE field this avenue of research specifically reported on interactions between genes and their polymorphisms, with hypothetical moderation of the effects of attachment security on social behavior (Hygen *et al.,* 2014).

Serotonin markers: 5HTTLPR

With regard to serotonin interactions, 5HTTLPR was examined in 4 papers, each reporting a significant association between the gene and behavior, when moderated by attachment status. Firstly, in relation to children with disorganized attachment, Zimmerman *et al.* (2009) reported that 5HTTLPR and attachment interacted to moderate aggressive behavior in adolescence. Specifically, children with the 5HTTLPR short (s/s or s/l) allele and classified as disorganized attachment exhibited more hostile autonomy and appeared more aggressive. Similar findings by

Kochanska *et al.* (2009) reported that children with the 5HTTLPR short (s/s or s/l) allele and disorganized attachment were more likely to develop poor self-regulation skills compared to children with the homozygous long (l/l) allele. Humphreys *et al.* (2015) added further support to this argument in their paper finding a significant association between the 5HTTLPR short (s/s), allele and disorganized attachment and an increased likelihood of displaying negative externalized behaviors. Finally, Viddal *et al.* (2017), reported findings for emotion regulation, demonstrating that children with the 5HTTLPR short (s/s) allele and disorganized attachment at 4 to 6 years were more likely to exhibit decreased emotion regulation from 6 to 8 years.

Furthermore, when observing those children within the above 4 studies classified as exhibiting a secure attachment, a similarly consistent pattern emerges. Zimmerman *et al.* (2009) reported that children with the 5HTTLPR short (s/s or s/l) allele and secure attachment exhibited more agreeable autonomy and appeared less aggressive. Kochanska *et al.* (2009) reported the same trends, with children with the 5HTTLPR short (s/s or s/l) allele and organized attachment demonstrating a likelihood to develop good self-regulation skills, compared to children with the long (l/l) allele. The results from Humphreys *et al.* (2015) also showed a significant association between children with the 5HTTLPR short (s/s) allele and secure attachment exhibiting less negative externalised behaviors. Finally, further support for this model comes from Viddal *et al.* (2017) who reported that children with the 5HTTLPR short (s/s) allele and secure attachment at 4 to 6 years were more likely to exhibit increased emotion regulation from 6 to 8 years.

Dopamine markers: DRD4, DRD2, COMT

With regard to dopamine, no studies examined the DRD4 or DRD2 genes in relation to GxE interaction on behavior. The COMT gene was highlighted in two studies (Hygen *et al.* 2014; Li *et al,* 2016*)*, both showing consistent, significant associations between the interaction of the gene, attachment status and externalized behaviors.

Hygen *et al.* (2014) reported findings that children with the COMT val/val allele and disorganized attachment were more likely to develop aggressive behavior and poor social skills compared to those children with the met allele. These findings were supported by Li *et al.* (2016) who reported that children with the homozygous val allele and disorganized attachment exhibited less positive and more negative behaviors than other children aged between 5 and 11. Li *et al.* (2016) classify this behavior as the 'punitive-controlling' sub-type of disorganized attachment. They also reported that children with the met alleles and disorganized attachment exhibited more positive and less negative behaviors than other children aged between 5 and 11 years. This was classified as the 'caregiving-controlling' sub-type of disorganized attachment.

Additional genetic markers

With regard to novel gene markers, Borrelli *et al.* (2017) reported that FKBP5 and attachment interact to predict externalized behaviors relating to emotion regulation. Their results show that child attachment security is inversely associated with respiratory sinus arrhythmia (RSA) reactivity, emotional suppression, rumination, and depressive symptoms among children with high

risk plasticity (CC allele), however there was no association found for children with the AA or AC allele. As discussed in previous sections, as these are de novo findings for candidate genes, no replication has yet been reported.

Study quality

Quality ratings were applied using a quantitative summing and a thematic overview of potential methodological sources of bias. With regard to overall score. within a possible score of 0 – 22, the scores across the studies ranged from 13 to 22. The most frequent issue reported for study methodology was reporting of sample size. The majority of the studies did not report the sample size calculations, making it difficult to ascertain whether power was sufficient. That said, many of the papers acknowledged this issue and addressed power and sample size within their discussion sections. A second issue noted across the papers was a tendency in some papers to inadequately address missing data or dropout over time. Thirdly, several papers did not report whether assessments were carried out blind to outcomes, although over half (n=16) did report blind testing. All genetic testing was carried out using validated methods, and testing for attachment was undertaken using validated methods, although it should be noted that 2 studies (Bakermans-Kranenburg *et al.,* 2011; Humphreys *et al.,* 2015) both used the SSP on older children. As a number of the papers derive from the same cohorts (Generation R, BIPS, TESS, MAVAN) the protocol followed by each paper was similar, if not identical. Summarizing across the included literature, while there are some methodological issues across the studies, with 4 papers receiving the top score of 22 (Gervai *et al.,*

2007; Hygen *et al.,* 2014; Raby *et al.,* 2012; Viddal *et al.,* 2017), it can be argued that this area of research has robust methodological procedures.

4. Discussion

To our knowledge, this review is the first to systematically summarize the literature on associations between genetic markers, environmental factors and attachment. **In doing so we have focused on infant and child attachment status**. The key findings of our review can be broken down into three main areas of interest; i) one-way associations between genetic markers and attachment (Gene as main effect), **where we find little evidence of consistent patterns of association**; ii) two-way associations between genetic markers, environment, and attachment (GxE interactions on attachment outcomes), **where we see some associations, albeit again somewhat inconsistently;** and iii) two-way associations between genetic markers, attachment, and behavior (GxE interactions with attachment as "E" on child outcomes), **where a clearer pattern emerges, particularly with regard to the role of attachment disorganization. A further methodological finding of our review is the as yet under-used potential of contemporary genomics to enrich the understanding of these patterns of association and interaction**.

When examining the impact of genetic markers upon attachment, we echo previous narrative reviews, indicating little consistency between findings. When considering 5HTTLPR, only 4 of the 14 studies that observed the marker even reported any significant associations, and these reports were not in agreement as to

the size of effect of the gene upon attachment. Similarly, with DRD4, only 5 of 12 studies showed any associations, with disparities between findings from different cohorts. Within the results, there was insufficient evidence of association to meaningfully comment on the direct influence of DRD2, OXTR, or COMT on attachment. We therefore support the contemporary position that genetic associations with attachment are most likely to emerge via interaction effects with environmental or behavioral variables (Bakermans-Kranenburg & van IJzendoorn, 2007).

Moving to our second set of findings, there seems to be a similar lack of consistency between reports of GxE interactions on attachment outcomes, **although some recurring patterns of association** do emerge. The existing literature is comparatively consistent on the putative influence of the serotonin gene 5HTTLPR in various environments, with a signal that 5HTTLPR long alleles were potentially implicated in secure attachment, and conversely that the short allele conferred increased risk of attachment disorganization. In contrast, findings for the main dopaminergic marker - DRD4 – appeared particularly inconsistent, with studies reporting **failure to replicate, or contradictory findings, for example**; Luijk, Roisman *et al.* (2011) reported contradictory findings **from two samples within the same study.** As with the direct associations, there are few significant interactions reported that implicated DRD2, OXTR, and COMT, making it difficult to comment on their effect upon attachment security.

Our final theme, which we propose **constitutes a potentially productive** pathway for future research in this area, considers the specific case of GxE

interactions between genetic markers and attachment in moderating child outcomes, particularly externalizing behaviors. In the included studies **(Borrelli *et al.*, 2017;** Zimmerman *et al.,* 2009; Kochanska *et al.* 2009; Hygen *et al.* 2014; Humphreys *et al.* 2015; Li *et al.* 2016; Viddal *et al.* 2017) there appears to be broad consistency amongst these results. **These** studies have so far mainly concentrated on the genes 5HTTLPR and COMT, **with one study considering novel genes such as FKBP5. However,** there is agreement amongst the results that **would appear to suggest that** these genes work in a regulated way to moderate behavior depending on the type of attachment classification that the child represents, and the environment in which the child finds him or herself. Therefore, for these genetic markers, there is evidence that the presence or absence of a particular allele will influence the child to exhibit more negative externalized behaviors if they also have disorganized attachment, whereas they will exhibit more positive externalized behaviors if they have a secure attachment with their primary caregiver. This is consistent with a differential susceptibility approach (Ellis *et al.* 2011), but also opens up the possibility that multiple gene interactions operate in synchrony to code for vulnerability or resilience in relation to the ontology of caregiver-child behavior. Therefore, future studies will need to both increase computational power to disentangle these associations, in tandem with further refinement of the use of biomarkers (e.g. Pappa et al., 2015).

Methodological considerations in genomics and attachment

There are a number of explanations that we propose could aid in understanding the lack of consistency that exists within the results. The inclusion and exclusion criteria for this review allowed studies that observed any candidate or de novo genetic marker in interaction with the environment. This increased the breadth of studies that we could include within the review. It could be argued from the key findings of our review that results for dopaminergic markers, particularly DRD4 and DRD2 are showing little consistency in their replicability across time, and it is possible to see shifts away from these candidate genes into **consideration of other biomarke**r systems. This is somewhat surprising given the frequent observation in infants of associations between DRD4 genes and the development of arousal and homeostatic regulation in the first 12 months of life (Papageorgiou & Ronald, 2016). However, it may be the case that these DRD4 associations are linked to nascent development of the infant's individual capacity to attend and regulate states, rather than the dyadic co-regulation of social interaction that we see in attachment behavior. Therefore, it is less that DRD4 is not implicated in attachment per se, but that DRD4 operates as a biological substrate within other regulatory systems, in tandem or overlapping with the attachment system. Our findings for GxE interactions support this notion, particularly for DRD4 and 5HTTLPR.

There is also the possibility that, as with genetic research in psychiatric disorder, we will see a process of research moving on "rapidly and essentially ad infinitum" (Roisman *et al.*, 2013, p385) as results from larger samples fail to replicate previous results. Our synthesis supports this with the DRD genes candidates, but also with genes in the oxytocin system, such as OXTR. Again, OXTR rapidly

emerged as a relatively new candidate gene implicated in attachment, given its association with social interaction (Bakermans-Kranenburg & van IJzendoorn, 2014; Leerkes *et al.,* 2017; Luijk, Roisman *et al.,* 2011; Roisman *et al.,* 2013). While there was interest in this gene, as part of a hormonal system linked to human social bonding (Carter, 1998) it has already been suggested that other than assisting in neonatal environments, such as childbirth and breastfeeding, there is little evidence to show that it is functional in developing external social behaviors (Bakermans-Kranenburg & van IJzendoorn, 2014; Bos, 2016). Furthermore, emerging lines of enquiry around both the use of intra-nasal OXT as a treatment in neurodevelopmental disorders and on endogenous OXT point to little evidence of consistent associations between OXT and social cognition (Kee, Crowe & Hocking, 2018). This pattern of identification of candidate gene x trait association, followed by lack of replicability presents something of a dilemma for developmental psychopathology (and the field of GxE interactions as a whole). A consensual approach may be to acknowledge the difficulty in observing genetic biomarkers in isolation and look to identification of multivariate associations as a more fruitful line of enquiry.

A further critique of the literature reviewed here is to suggest that the studies included have such a breadth of heterogeneous variables that between-study comparisons are hampered from the start. For instance, while a number of the studies observe the interaction between genetic marker and maternal sensitivity on attachment, still other studies chose to observe the interaction between the gene and a different variable within the child's environment. The challenge is therefore both a methodological and theoretical one. From a methodological standpoint this broad

spectrum of covariates, and variation in measures within variables (e.g sensitivity) introduces error and bias and could inflate the risk of contradictory findings. From a theoretical standpoint, the lack of consistency between studies which evaluate the same environmental variable, also points to the presence of additional factors presently unaccounted for in existing models. Successful modelling of these associations therefore requires construction of larger samples, evoking the international consortia assembled for the large-scale analyses in psychiatric genetics (e.g. Milaneschi *et al.*, 2017). As noted earlier in our discussion, we also suggest that clearer reporting as to whether GxE studies consider attachment as the outcome, or as the "E" in a GxE interaction would also aid future research in establishing replicable patterns of association.

Considerations for theoretical frameworks involving attachment and genetics

If we take the aforementioned complexity as a given, we can then reformulate the GxE problem in attachment via classic developmental psychopathology concepts of multifinality and equifinality (Cicchetti & Rogosch, 1996). Even when children start out with seemingly the same environmental and biological factors, they may, none-the-less, develop along different trajectories; or conversely, may find themselves travelling along the same developmental pathway from very different starting points. This tension can be observed in the contradictory interpretations of the recent meta-analysis of the transmission gap (Verhage *et al*. 2016, 2017; Barbaro *et al*. 2017). Our review thus suggests that gene-environmental interaction could be considered the baseline for enquiry into the role of genetics in attachment, and that

we need to attend more clearly to how we parse and delineate the environmental variables in these models.

The findings in the current review give strong support to the importance of differential susceptibility as an explanatory framework within which associations between genes, attachment, and other developmental factors can be understood (Bakermans-Kranenburg & van IJzendoorn, 2007; 2019). As previously discussed, differential susceptibility theory argues that a specific gene (**or a composite of multiple genes combined into a polygenic susceptibility score (Belsky & Beaver, 2011)),** may serve as a risk factor when exposed to a negative environment, but conversely, may enhance development when placed in optimal conditions. This is most easily discerned within the studies observing the interaction between genetic marker and attachment, moderating externalized behaviors. One potential implication of our review is that this pattern of associations, aligned to concepts of multifinality, equifinality, and differential susceptibility, pave the way for future genetics of attachment studies to take advantage of epigenetics – the dynamic process whereby genes respond to the experiences within the child's environment (Dudley, Li, Kobor, Kippin, & Bredy, 2011). Epigenetic expression is a complex system through which interactions may lead to a number of different phenotypic, and behavioral, outcomes (Bos, 2016). Emerging findings suggest that incorporating variance epigenetic processes into standard GxE models of attachment and developmental outcomes may clarify patterns of GxE association (Meaney, 2010). For instance, findings from the Generation R cohort suggest that FKBP5 methylation (an epigenetic binding protein associated with HPA axis function) moderates the

associations between the FKBP5 genotype and resistant attachment with cortisol reactivity (Mulder *et al*., 2017). Findings such as these lead us to understand the interaction between gene and environment, not as a simple model (Fig.2), but as complex modelling in which factors are not only influencing each other in a determined way, but are at the same time being impacted upon in a dynamic nature (Fig.3) (Champagne, 2016). Future studies may therefore wish to augment consideration of the Genetic aspects of GxE interactions on attachment through consideration of genome wide epigenetics.

Limitations

These implications notwithstanding, there are a number of limitations of the review that should be highlighted in order to gain a greater understanding of the results contained within. One methodological issue that may need to be addressed in future studies is the measurement of attachment which is used. Verhage *et al.* (2016) suggest that by using different measures of attachment; ie. dimensional vs categorical, this may change the operationalization of the measurement. The authors also question the inter-reliability of measuring attachment at different ages, using different tools.

While there are **27** studies assessed in the review, these are representative of only **16** cohorts. This leads to an over representation of some of the cohort samples for information; especially the Generation R cohort (which is appraised 4 times within the review). As these cohort studies use the same population samples repeatedly, and are only slightly manipulating the environmental variables, there is

likely to be a strong concurrence between their results each time. While this may lead to a results table which appears to lend convincing support for one argument, it is important to bear in mind that each cohort could be considered as only one result; indicating weaker results than are presented currently. Additionally, small sample sizes used in a number of the studies could create underpowered work from which it is difficult to extrapolate meaningful results. Leading on from this is the number of genetic markers that were measured against environmental influences. Roisman *et al.,* (2013) have argued that with the sheer number of genetic markers that could be nominated as candidate genes, combined with the tendency to use smaller, underpowered, samples there is more scope for researchers to report significant associations where they may not appear in larger study samples.

Given the heterogeneity of measurement variables and methodologies we **suggest that there is yet insufficient numbers and homogeneity within published studies to justify a meta**-analysis. **However, it would lend more strength to the presented results to be able to statistically analyze outcomes across studies and it seems a reasonable proposition that this will become a viable approach in the near future. However, we note that synthesis of these results** is compounded by variations in the measurement of genetics (e.g. different variants of dopamine markers including DRD4, DRD2, and COMT) which further reduce comparability of samples. We also note that the small number of studies for each outcome introduces risk of small study effects on any meta analytical estimates. As it stands, this literature does not seem homogenous enough to sustain a meta-analysis. However,

as contemporary GxE studies accumulate, it would be viable to synthesize these results via meta-analysis.

Implications for research

The review's findings generate a number of implications for research, which we propose could **influence the next generation of GxE studies involving attachment and in some instances these are already being enacted**. As underpowering and reliance on small studies is a key weakness of the literature, it would be advisable that more cohorts work together to increase the power of their sample sizes, as evidenced by domains such as psychiatric genetics research (Papageorgiou & Ronald, 2017). Parallels can be drawn **with other areas of research from development, through to behavioral economics, where genomics is increasingly being used to leverage understanding of the links between GxE and behavioral outcomes. This also lends itself to the types of complex modelling that are already widespread in the field of developmental psychopathology.**

A further, complementary avenue of research is to explore and model the longitudinal effect of genetic biomarkers and their interaction with dynamic changes in the environment in which the child is developing (Bakermans-Kranenburg & van IJzendoorn, 2011). **As noted above, epigenetics is not a deterministic factor, but instead operates as a dynamic system that constantly affects the individual from childhood and onward** throughout the life course (van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010). It may also be suggested that the genotypes within the child's system are influencing behavior at different ages throughout development, which in turn would influence the mechanisms of

developing attachment security (Drury *et al.,* 2012; Papageorgiou & Ronald. 2017). A longitudinal study would be more effective in detecting such influences across time. Equally, in addition to methylation, there are other potential epigenetic markers that could be explored in relation to attachment including chromatin structure and noncoding RNA (Gartstein & Skinner, 2017). In taking this approach, there is the potential to link attachment research to the burgeoning field of developmental origins of health and disease (the DoHaD hypothesis; Wadhwa, Buss, Entringer & Swanson, 2009; O'Donnell & Meaney, 2016). Other options to delineate longitudinal associations would be to incorporate experience sampling methodologies into measurement of behavioral or caregiving variables, as has been successfully demonstrated in research into at risk psychopathologies in young adults (Myin-Germeys *et al.* 2009)

Leveraging new and emergent technologies for genomic and epigenomic research may also open additional opportunities for developmental psychopathology. While many of the studies included within the current review focused on specific genes, there is evidence to suggest that observing gene x gene interactions could produce meaningful findings; as demonstrated by Cicchetti *et al.,* (2011) who report that the significant interaction between DRD4 and 5HTTLPR influenced attachment organization, rather than one gene candidate or another. Similarly, Pappa *et al.* (2015) also argue that the genetic substrate of the endophenotype of disorganized attachment may be the result of multiple genes of small effect working in concert. The use of genome-wide association study (GWAS) technologies could shed light on these gene-to-gene interactions, but also elucidate

further epigenetic pathways that may impact on attachment downstream from the genetic action. These would include additional factors implicated in synaptic transmission. Rather than specifying a priori genetic markers, GWAS is a bottom-up approach, using computational modelling to survey the whole genome to identify potential genetic markers that associate with the outcome variable. Papageorgiou and Ronald (2017) argue that by using this method of genetic testing, the researcher does not have to hypothesize about the mechanisms of one specific gene, and instead allows a broader view into the effects that multiple genes are enacting upon each other. The application of this methodology in the Pappa *et al.* (2015) study demonstrates proof of concept to identify novel genes, and their pathways associated to both disorganization and attachment security. This type of broad, dynamic study would allow researchers to understand the influence of multiple genetic, neural, and environmental factors working at the same time.

In summary, the current review and evidence synthesis demonstrates the **change in the complexity of research in genetic markers for attachment over the last 20 years, from investigation of main effects of genes on attachments, to a contemporary position that situates gene markers and attachment within complex systems models, where attachment may constitute an outcome in interaction with another environmental factor or factors, or may itself constitute an environmental factor in relation to other developmental outcomes. In both cases, these GxE interactions can at present be best understood within a differential susceptibility model of development. Given the heterogeneity of outcomes noted in our review there is still a need for further research into the field of attachment organization and modelling of the** complex systems that are involved in delineating the

longitudinal unfolding of the attachment system. **This includes modeling of the factors contributing to the transmission gap. Our findings also support the need for more robust approaches as to how we conceptualize and measure gene markers in relation to attachment, which emerges as a key methodological finding of our review. For the field to keep pace with other genomic research endeavors (such as contemporary psychiatric genetics) w**ill require larger samples and new approaches to explore the contribution of novel genes, suggestive chromosomal loci, cation transport, synaptic pathways, GWAS and single nucleotide polymorphism; in interaction with a child's environment. We propose that application of these new approaches and the advent of probabilistic **epigenetics offer significant opportunities for** developmental psychopathology researchers to improve their understanding of how genes and environment interact throughout the life course to influence, and be influenced by attachment.

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Table 1. Characteristics of included studies

Cohort	Authors, Year	Location	Sample; *N,* age, gender	Measurement of attachment	Genes specified	Environmental factors considered
BEIP	Humphreys *et al.* 2015	Romania	*N = 106* 54 months old	SSP (42 months)	5-HTTLPR	Early institutional care Attachment mediating externalizing behavior
BIPS	Lakatos *et al.* 2000	Budapest	*N* = 90 12 months' old m = 52 f = 38	SSP (12 months)	DRD4 (exon III 48-bp repeat polymorphism)	*Not evaluated*
	Lakatos *et al.* 2002	Budapest	*N* = 95 12 months' old m = 54 f = 41	SSP (12 months)	DRD4 (exon III 48-bp polymorphism and -521 C/T snp)	*Not evaluated*
	Gervai *et al.* 2005	Budapest	*N* = 95 12 months' old m = 54 f =41	SSP (12 months)	DRD4 (exon III 48-bp polymorphism and -521 C/T snp)	*Not evaluated*
BIPS / Harvard Medical	Gervai *et al.* 2007	Budapest / USA	<u>BIPS cohort</u> *N* = 96 m = 55 f = 41 <u>Harvard Medical cohort</u> *N* = 42	SSP (BIPS – 12 months Harvard – 18 months)	DRD4 (exon III 48-bp repeat polymorphism) 5-HTTLPR (polymorphism)	Parental disrupted communication

	Brumariu *et al.* 2016	Budapest / USA	*N* = 39 12-18 months' old m = 20 f = 19	SSP (12-18 months)	5-HTTLPR (rs25531) s/s - 8 s/l - 18 l/l - 13	Maternal behavior Infant proneness to distress during SSP
California	Borelli *et al.* 2017	USA	*N* = 99 9-12 years' old m = 51 f = 48	Security Scale	FKBP5	Maternal overcontrol Child emotion suppression Child rumination Child depressive symptoms
Duke University	Propper 2006	USA	*N* = 169 12 months' old m = 85 f = 84	SSP (12 months)	DRD2 (A1/A1, A1/A2, A2/A2), DRD4 (-521 T/T, T/C, C/C), 5-HTTLPR (s/s, s/l, l/l)	Maternal sensitivity Maternal negativity
GEM	Bosmans *et al.* 2018	USA	*N* = 487 7-16 years' old	ECR-RS (7-16 years)	NR3C1 methylation	Chronic stress severity Longitudinal stress exposure Maternal support Depressive symptoms Externalizing problems

Generation R Cohort participants born between 2003 - 2005	Luijk *et al.* 2010	The Netherlands	*N* = 310 14 months' old m = 175 f = 135	SSP (14 months)	Polymorphisms in the glucocorticoid receptor gene, BclI (rs41423247), TthIIII (rs10052957), GR-9b (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190) FKBP5 gene (rs1360780)	Stress Reactivity during SSP
	Luijk, Tharner *et al.* 2011	The Netherlands	*N* = 601 14 months' old m = 308 f = 293	SSP (14.7 months)	Glucocorticoid receptor gene, BclI (rs41423247), TthIIII (rs10052957), GR-9 (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190); Mineralocorticoid receptor gene (rs5522).	Maternal sensitive responsiveness Maternal extreme insensitivity
	Pappa *et al.* 2015	The Netherlands	*N* = 657 14 months' old	SSP (14 months)	HDAC1	Attachment style during SSP

			m = 335 f =322		ZNF675 BSCD1 CACNA2D3	
Generation R / SECCYD	Luijk, Roisman *et al.* 2011	The Netherlands / USA	<u>Generation R cohort</u> *N* = 663 m = 345 f = 318 <u>SECCYD cohort</u> 1,191 m = 572 f = 619	SSP (15 months)	DRD4 (48 bp VNTR) DRD2 (rs1800497) COMT Val158Met (rs4680), 5-HTTLPR OXTR (rs53576 and rs2254298).	Maternal sensitivity Mother-child interactions
Leiden	van IJzendoorn & Bakermans-Kranenburg 2006	The Netherlands	*N* = 85 14-15 months old m = 46 f = 39	SSP (14-15 months)	DRD4 (7-repeat allele and -521 C/T snp)	Maternal unresolved loss / trauma Maternal frightening behavior
MAVAN Data collected	Graffi *et al.* 2015	Canada	*N* = 251 T1 3 months' old T2 6 months' old T3 12 months' old T4 18 months' old	Modified SSP (36 months)	DRD4 (7 repeat allele)	Birthweight

between 2003 - 2009			T5 yearly assessments from 24 months m = 115 f = 116			
	Graffi *et al.* 2018	Canada	*N* = 655 T1 3 months' old T2 6 months' old T3 12 months' old T4 18 months' old T5 yearly assessments from 24 months m = 355 f = 300	Modified SSP (36 months)	DRD4 (7 repeat allele)	Early maternal care using Ainsworth Maternal Sensitivity Scale. Maternal Depression
Minneapolis Health Department Participants recruited between 1975 – 1977	Raby *et al.* 2012	USA	*N* = 154 6-18 months' old m = 74 f = 81	SSP (12 and 18 months)	5-HTTLPR (tri- allelic genotype)	Maternal responsiveness
Mount Hope Family Centre	Cicchetti *et al.* 2011	USA	*N* = 152 *106 from maltreating families* *47 from non-maltreating families* 13 months' old	SSP (12 and 24 months)	5-HTTLPR DRD4 (exon III variable number tandem repeat) DRD4	Maltreated vs non-maltreated children Preventative interventions used for maltreated children between 12 and 24 months

					(-521 C/T snp)	
Regensburg Longitudinal Study data collected between 1974 - 2005	Spangler *et al.* 2009	Germany	*N* = 106 12 months' old m = 53 f = 53	SSP (12 months)	DRD4 (exon III repeat polymorphism) 5-HTTLPR (polymorphism and -521 C/T snp)	Maternal sensitivity
	Zimmerman *et al.* 2009	Germany	*N* = 91 12 years' old m = 45 f = 46	LCAI (12 years)	5-HTTLPR (short allele)	Socially evaluative context to elicit adolescent fear Attachment mediating emotion regulation
SECCYD	Li *et al.* 2016	USA	*N* = 560 15 months' old m = 275 f = 285	SSP (15 months)	COMT (Val158met Val/Val, Val/Met, Met/Met)	Attachment mediating aggressive behavior Social competence
TESS data collected between 2007 - 2011	Hygen *et al.* 2014	Norway	*N* = 704 4 years' old m = 359 f = 345	MCAST (4 years)	COMT (Val158met)	Attachment mediating aggression Social skills
	Viddal *et al.* 2017	Norway	*N* = 678 T1 4 years' old T2 6 years' old T3 8 years' old	MCAST (4 years and 6 years)	5-HTTLPR (s/s - 18.4% s/l - 51.5% l/l - 30.1%)	Attachment mediating emotion regulation
Ukraine	Bakermans-Kranenburg *et al.* 2011	Ukraine	*N* = 37 *18 reared in care homes*	SSP	5-HTTLPR (s/s, s/l vs l/l allele)	Institutionalized care

			19 family reared in biological parents' home 3-6 years' old			
University of Iowa	Barry *et al.* 2008	USA	*N = 89* 7-52 months' old m = 40 f = 49	SSP (15 months)	5-HTTLPR (s/s s/l vs l/l allele)	Mother's responsiveness
	Kochanska *et al. 2009*	USA	*N = 88* 7-52 months' old m = 44 f = 44	SSP (15 months)	5-HHTLPR (s/s s/l vs l/l allele)	Attachment mediating self-regulation in effortful control tasks
University of North Carolina	Leerkes *et al.* 2017	USA	*N* = 200 6-12 months' old m = 96 f = 104	SSP (12 months)	DRD2 DRD4 COMT, 5HTTLPR (biallelic and triallelic) OXTR	Maternal behavior and sensitivity

Abbreviations: BEIP: Bucharest Early Intervention Project, BIPS: Budapest Infant-Parent Study, ECR-RS: Experiences of Close Relationships-Relationship Structures Questionnaire, GEM: Gene Environment Mood Study, LCAI: Late Childhood Attachment Interview, MCAST: Manchester Child Attachment Story Task, MAVAN: Maternal Adversity, Vulnerability and Neurodevelopment Project, SSP: Strange Situation Procedure, SECCYD: Study of Early Child Care and Youth Development, TESS: Trondheim Early Secure Study

Table 2 – Results for associations genetic / gene x environment and organized attachment (Secure, Avoidant, Anxious Ambivalent)

Authors, Year, Cohort	Main findings	Results for gene attachment association	Results for GxE interaction on attachment	Covariates identified
Infants (0-18 months)				
(i) Gervai *et al.* 2005 BIPS	DRD4 7-repeat allele less frequently transmitted to infants with secure attachment. Absence of T.7 haplotype of DRD4 gene is a resilience factor for the development of early attachment	Significantly lower-than-expected transmission of DRD4 7-repeat allele to securely attached infants from parents. **TDT_X2 = 6.00, df = 1, *P* = 0.014**	Not evaluated	Significant association for non transmission of the T.7 haplotype **TDT_X2 = 4.455, df = 1, *P* = 0.035**
(ii) Propper 2006 Duke University	Association between DRD2 and 5HTTLPR and avoidant attachment behavior Significant association between avoidant attachment behaviour and 5HTTLPR l/l allele when exposed to negative parenting	Significant association for 5HTTLPR and avoidant attachment during episode 8 of SSP **F(2, 84) = 3.67, *P* = *.03*** Significant association for DRD2 and avoidant attachment during episode 8 of SSP **F(2, 84) = 3.23, *P* = *.045***	Significant association between avoidant attachment and 5HTTLPR (l/l) allele in episode 5 of SSP when exposed to negative parenting **ß = .433, Õx = .17, t = 2.58, *P* = .037**	

(iii) Barry *et al.* 2008 Uni. of Iowa	5-HTTLPR short allele (ss/sl) at risk for disorganized attachment when also exposed to unresponsive maternal care No significant association for infants with short allele and responsive care	Significant association between 5HTTLPR and attachment security **b = 1.54, SE = .54, *P*= < .01**	Significant interaction of 5HTTLPR genotype with mother responsiveness, **b = –1.76, SE =.90, *P* = <.05**	s/s & s/l allele; responsiveness significantly positively predicted attachment security **odds ratio = 2.46, *P* = < .01** l/l allele; responsiveness not associated with attachment organization **odds ratio=.40, ns**
(iv) Luijk *et al.* 2010 Generation R	No significant association for genetic impact on attachment security. Significant association between FKBP5 and resistant attachment impacting on cortisol stress reactivity	No significant association	Not evaluated	Significant association between FKBP5 and resistan attachment impacting on cortisol stress reactivity **β =.12, *P* <.05**
(vi) Luijk, Roisman *et al.* 2011 Generation R / SECCYD	No consistent evidence to associate genes DRD2/4, 5HTTLPR, COMT, OXTR to attachment security	Significant association with 5HTTLPR short allele and attachment security in Generation R sample. ***P* = 0.04** Results not significantly replicated in SECCYD sample	Significant association between absent DRD4 7-repeat allele and attachment security in the presence of parental sensitivity in SECCYD sample ***P* = .004** Opposite trend reported in Generation R sample,	Significant associations between breastfeeding and attachment security (p < .01), genotype (p < .05), and maternal sensitivity (p < .01). No adjustment made to final results with specified covariat

(v) Luijk, Tharner *et al.* 2011 Generation R	Significant association between infants carrying the minor MR allele (G) and secure attachment in the presence of mother's responsiveness, however significantly less securely attached if exposed to unresponsive maternal care	No significant association	Minor MR allele (G) x mother's sensitive responsiveness increases secure attachment **β =.22, *P* =.02** Minor MR allele (G) x insensitive unresponsiveness reduces secure attachment **β = −.29, *P* = < .01**	
(vii) Raby *et al.* 2012 Minneapolis	No significant associations between genetic impact and attachment security. Association between mother's responsiveness and attachment security.	No significant association	No significant association	Significant association between mother's responsiveness and secure attachment in infants categorized as low distress during SSP aged 12 months ***OR* = 1.54, *P* = .01** Significant association between mother's responsiveness and secure attachment in infants categorized as high distress during SSP aged 18 months ***OR* = 1.50, *P* = .05**

(viii) Pappa *et al.* 2015 Generation R	Significant association between novel gene BECN1 and attachment security	BECN1 novel gene significantly associated with attachment security (Bonferroni-corrected threshold, p < 2.80e-06). ***P* = 2.00e-06**	Not evaluated	
(ix) Brumariu *et al.* 2016 BIPS/Harvard cross cultural	No significant association between 5HTTLPR and secure/insecure attachment No significant association for 5HTTLPR, attachment security and mother's responsiveness	No significant association	No significant association	Infant proneness to distress significantly associated to 5HTTLPR (***P* = < .05)**
(x) Leerkes *et al.* 2017 North Carolina	Little evidence to link candidate genes DRD2, DRD4, COMT, biallelic and tri-allelic 5HTTLPR, and OXTR with attachment security or disorganization, or when exposed to maternal	No significant association	Significant association between DRD4 and attachment security when exposed to maternal sensitivity (not moderated by race) **β = −.19, *P* = .05**	Significant association between 5HTTLPR (biallelic) and attachment security when accounting for race **β = .26, *P* = .05**

	sensitivity / negative behavior		Significant association between OXTR and attachment security when exposed to maternal sensitivity among African-American infants, **β = −.29, *P* = .05** Association predicts higher attachment security in infants without OXTR risk allele	No significant association between candidate genes and attachment security when exposed to maternal negative behavior
Early Childhood (18-52 months)				
(xi) Cicchetti *et al.* 2011 Uni. of Rochester	Intervention improved attachment security in maltreated children. Genetic variation did not influence improvement in attachment security in maltreated children but DRD4 and 5HTTLPR influenced improvement in attachment security in nonmaltreated children	No significant association between 5HTTLPR s/s s/l or l/l alleles and attachment classification	No significant association between 5HTTLPR s/s s/l l/l allele and secure attachment in maltreated children regardless of intervention Significant association between 5HTTLPR l/l allele and secure attachment in nonmaltreated children **x^2 (1, N = 42) = 6.42, *P* = .025**	No significant differences reported in presence of DRD4 across racial / ethnic groups Significant difference reported in presence of 5HTTLPR (s/s or s/l allele) in black children compared to white or multiracial/other racial / ethnic groups **x^2(2, N = 153) =11.42, *P*=.003**

		No significant association between DRD4 and attachment classification	No significant association between DRD4 and secure attachment in maltreated children regardless of intervention Significant association between DRD4 and secure attachment in nonmaltreated children **x^2 (1, N = 40) = 7.30, *P* = .013**	
Childhood to Adolescence (5-18 years)				
(xii) Borelli *et al.* 2017 California	No significant association for interaction between FKBP5 and maternal overcontrol on secure/insecure attachment	Not evaluated	No significant association	
(xiii) Bosmans *et al.* 2018 GEM	No significant association for children with NRC31 methylation, low maternal support and avoidant attachment. Significant association to predict that children with anxious attachment,	Not evaluated	In children with anxious attachment NRC31 methylation x low maternal support = higher anxious attachment in the context of higher stress levels **p=0.0001**	

	NRC31 methylation, and low maternal support demonstrate higher anxious attachment in the context of higher stress levels			

Abbreviations: BIPS: Budapest Infant Parent Study, GEM: Gene Environment Mood Study, RLS: Regensburg Longitudinal Study, SSP: Strange Situation Procedure, SECCYD: Study of Early Child Care and Youth Development, TESS: Trondheim Early Secure Study

Table 3 – Results for associations genetic / gene x environment impact and disorganized attachment

First Author, Year, Cohort	Main findings	Results for gene attachment association	Results for GxE interaction on attachment	Covariates identified
Infants (0-18 months)				
(i) Lakatos *et al.* 2000 BIPS	DRD4 7-repeat allele found with significantly higher frequency in infants with disorganized attachment	DRD4 7-repeat genotypes significantly differentiated between disorganized and non-disorganized population **$x^2 = 8.66$, df = 1, $P < 0.005$**	Not evaluated	No difference when accountir for gender Boys: **$x^2 = 6.03$, df= 1, P 0.0** Girls: **$x^2 = 4.51$, df = 1, P 0.0**
(ii) Lakatos *et al.* 2002 BIPS	Association between DRD4 7-repeat allele and disorganized attachment significantly enhanced by -521 T allele.	Presence of DRD4 and -521 T DRD4 7-repeat allele significantly increases risk of disorganized attachment **$x^2 = 6.61$ & 6.67, df =1, P = 0.025** (for CT and TT genotypes, respectively)	Not evaluated	

(iii) Gervai *et al.* 2005 BIPS	Disorganized attachment linked to the DRD4 7-repeat allele.	Significantly higher transmission of DRD4 7-repeat allele to disorganized infants **$TDT_X2 = 3.27$, df = 1, *P* = 0.071**	Not evaluated	
(iv) van IJzendoorn & Bakermans-Kranenburg 2006 Leiden	Results show 18.8 fold increase in disorganized attachment in children with DRD4 7-repeat allele when also exposed to maternal unresolved loss or trauma.	No significant association	Significant association between disorganized attachment and DRD4 7-repeat allele when also crossed with maternal age and maternal unresolved loss/trauma **F(6, 56) = 2.83, p = .02.** No significant associations between DRD4 7-repeat allele when exposed to maternal frightening behavior	Significant association with maternal age **(beta = .32, p = .01)** Significant association with maternal unresolved trauma/loss **(beta = .29, p = .02, odds ratio 2.98)** No significant associations fo -521 C/T allele
(v) Gervai *et al.* 2007 BIPS / Harvard cross cultural	Significant association between DRD4 short form allele and disorganization when exposed to maternal disrupted communication. No significant association for DRD4 7-repeat allele and disorganization in	No significant association	Significant association between DRD4 and disorganized attachment when exposed to maternal disrupted communication **t(133) = -2.18, *P* = .03, B = .35** Significant association between DRD4 short form	No significant association between DRD4 7-repeat allel disorganized attachment and gender Significant association between disorganized attachment and maternal disrupted communication

	relation to maternal communication		(*without 7-repeat allele*) and disorganized attachment when exposed to maternal disrupted communication **t(87) = 4.35, *P* = <.0001, B =.37**	**t(134) = 3.18, *P* < .002, B= .3** Significant association between DRD4 and disorganized attachment whe exposed to maternal disrupte communication when demographic risks controlled **t(133) = 2.10, *P* =<.04, B= .6**
(vi) Spangler *et al.* 2009 RLS	Significant association between 5HTTLPR s/s s/l allele and disorganized attachment. Indication that association is related to low maternal responsiveness	Significant association between 5HTTLPR genotype and disorganized attachment **linear x_2 (2, N = 96) = 6.57, *P* = .02**	Significant association between 5HTTLPR and disorganized attachment when exposed to poor maternal responsiveness **F(2,89) = 3.58, *P* = .03, x_2 = .07** Significantly higher proportion of infants classified as disorganized with s/s allele **P = < .05**	No significant association between infant genotype and maternal behavior
		No significant association between DRD4 and disorganized attachment	No significant association between DRD4 and disorganized attachment when exposed to poor maternal responsiveness	
(vii) Luijk, Roisman *et al.* 2011 Generation R /	No consistent evidence to associate genes DRD2/4, 5HTTLPR, COMT, OXTR	No significant association	Significant association between COMT and attachment disorganization in the presence of parental	

SECCYD	to attachment disorganization		sensitivity in Generation R sample ***P* = .04**	
(viii) Pappa *et al.* 2015 Generation R	Significant association between novel genes HDAC1, ZNF675, BSCD1 and pathways, and attachment disorganization	Genes significantly associated with attachment disorganization (Bonferroni-corrected threshold, p < 2.80e-06). HDAC1: ***P* = 1.00e-06** ZNF675: ***P* = 1.00e-06** BSCD1: ***P* = 2.00e-06**	Not evaluated	
(ix) Brumariu *et al.* 2016 BIPS/Harvard cross cultural	No significant association between 5-HTTLPR and disorganized attachment No significant association for 5-HTTLPR, attachment security and mother's responsiveness	No significant association	No significant association	Infant proneness to distress significantly associated to 5HTTLPR (***P* = < .05)**

(x) Leerkes *et al.* 2017 North Carolina	Little evidence to link candidate genes DRD2, DRD4, COMT, biallelic and tri-allelic 5HTTLPR, and OXTR with attachment security or disorganization, or when exposed to maternal sensitivity / maternal negative behavior	Significant association between OXTR and disorganized attachment (not moderated by race) **r = .18, *P* = .01**	No significant association	No significant association between candidate genes an disorganized attachment whe exposed to maternal negative behavior
Early Childhood (18-52 months)				
(xi) Bakermans-Kranenberg *et al.* 2011 Ukraine	Short allele of 5-HTTLPR (s/s & s/l) at higher risk of disorganized attachment when brought up in institutionalized care. Long allele (l/l) provides protective factors against adverse environmental factors	No significant association	Interaction between 5HTTLPR and type of care significantly predicted attachment disorganization ***F*(1,32) = 4.54, *P* = .04,** s/s & s/l alleles significantly more at risk when placed in institutionalized care compared to family home **t(23) = 3.48, *P* = < .01, d = 1.45**	
(xii) Cicchetti *et al.* 2011 Uni. of Rochester	Intervention improved attachment security in maltreated children. Genetic variation did not influence improvement in attachment security in	No significant association between 5HTTLPR s/s s/l or l/l alleles and attachment classification	No significant association between 5HTTLPR s/s s/l or l/l alleles and disorganized attachment in maltreated children regardless of intervention	No significant differences reported in presence of DRD4 across racial / ethnic groups Significant difference reported in presence of 5HTTLPR (s/s

	maltreated children but DRD4 and 5HTTLPR influenced improvement in attachment security in non-maltreated children		Significant association between 5HTTLPR s/s s/l alleles and disorganized attachment in non-maltreated children **x^2 (1, N = 42) = 6.22, *P* = .03**	or s/l allele) in black children compared to white or multiracial/other racial / ethnic groups **x^2(2, N = 153) =11.42, *P*=.00**
		No significant association between DRD4 and attachment classification	Significant association between absence of DRD4 and disorganized behavior in maltreated children before positive intervention **x^2 (1, N = 48) = 7.20, *P* = .017** No significant association reported after intervention. No significant association reported in maltreated children control group (no intervention) at baseline or follow up Significant association between DRD4 and disorganized attachment in non-maltreated children **x^2 (1, N = 40) = 5.63, *P* = .04,**	

		No significant associations for DRD4 and 5HTTLPR combined risk genotypes	Significant association for DRD4 and 5HTTLPR combined risk genotypes and disorganized attachment in non-maltreated children **x^2 (1, N = 40) = 9.82, *P* = .003**	
(xiii) Graffi *et al.* 2015 MAVAN	Children without DRD4 7-repeat allele more likely to exhibit disorganized attachment	Significant negative effect on disorganized attachment when DRD4 7-repeat allele present **b =-1.196, t(230)= 0.411, *P*=0.004**	No significant association	No difference when accountir for gender Maternal education status associated with disorganized attachment **χ2 (DF = 2, N = 231) = 18.99, p = .000**
(xiv) Graffi *et al.* 2018 MAVAN	Significant association between DRD4 and disorganized attachment	Significant association between DRD4 and disorganized attachment. DRD4 7-repeat allele predicted less disorganized attachment **β = - 1.11, OR = 0.333,** ***P* = 0.0008**	No significant association	Chronic maternal depression significantly predicted disorganized attachment **β = 1.01, OR = 2.74,** ***P* = 0.00911** Maternal education status associated with disorganized attachment college level, **β = -1.76, OR = 0.173, *P* = 0.0000928,** university level or higher,

				β = -1.15, OR = 0.316, *P* = 0.00284

Abbreviations: BIPS: Budapest Infant Parent Study, MAVAN: Maternal Adversity, Vulnerability and Neurodevelopment Project, RLS: Regensburg Longitudinal Study, SECCYD: Study of Early Child Care and Youth Development, TESS: Trondheim Early Secure Study

Table 4 – Results for associations gene x environment impact (attachment classification) and externalized behaviors.

Authors, Year, Cohort	Findings	Results for GxE interaction on externalized behaviors	Covariates
(i) Zimmerman *et al.* 2009 RLS	5HTTLPR and attachment interact to moderate aggressive behavior in adolescence. Children with s/s or s/l allele and disorganized attachment exhibited more hostile autonomy and appeared more aggressive. Children with s/s or s/l allele and secure attachment exhibited more agreeable autonomy and appeared less aggressive.	Significant association between high risk alleles (s/s or s/l) and autonomy when exposed to secure attachment: More agreeable autonomy **t(25.9) = -3.1, *P* = .005** Less hostile autonomy t**(43.8) = 3.9, *P* < .0001**	
(ii) Kochanska *et al.* 2009 Uni. of Iowa	5HTTLPR and attachment interact to determine self-regulation capacities from early childhood to middle childhood Children with s/s or s/l allele and disorganized attachment more likely to develop poor self-regulation skills.	Significant association between high risk alleles s/s & s/l, and self-regulation when attachment classification accounted for **b = 1.18, SE = .50, *P* < .02**	No changes to associations when accounting for gender

	Children with s/s or s/l allele and organized attachment more likely to develop good self-regulation skills (comparable to those of l/l allele)		
(iii) Hygen. *et al.* 2014 TESS	COMT and attachment interact to moderate aggressive behavior and social competence. Children with Val/Val allele and disorganized attachment more likely to develop aggressive behavior and poor social skills compared to children with Val/Met or Met/Met allele	Significant association between genetic marker and aggression; **Dx2 = 13.61 df =1, *P* = .0002** other-oriented social skills; **Dx2 = 9.19, df =1, *P* =.002** self-oriented social skills; **Dx2 = 7.80, df = 1, *P* = .005** (when exposed to disorganized attachment at age 4) Age 4-6 most disorganized attached (HighD) children showed decrease in aggression, children with Met allele showed greatest decrease and children with Val allele showed smallest decrease **x2 (1) = 7.13, *P* = .008**	
(iv) Humphreys *et al.* 2015 BEIP	5HTTLPR and attachment interact to predict negative externalized behaviors later in childhood. Children with s/s allele and disorganized attachment more likely to exhibit negative externalized behaviors. Children with s/s allele and secure attachment less likely to exhibit negative externalized behaviors.	Significant association between genotype (s/s vs s/l vs l/l) and negative externalized behaviors at 54 months when exposed to disorganized attachment at 42 months **F(2,21) = 4.20, *P* = .03** Significant association between s/s allele and negative externalized behaviors at 54 months when exposed to disorganized attachment at 42 months **18.04 [3.39] *P* < .02**	

(v) Li *et al.* 2016 SECCYD	COMT and disorganized attachment interact to determine externalized behaviors Children carrying Met alleles and disorganized attachment exhibit more positive / less negative behavior than other children aged 5 and 11 (caregiving-controlling style) Children with homozygous Val alleles, and disorganized attachment exhibit less positive / more negative behavior than other children aged 5 and 11 (punitive-controlling style)	Significant association between Met carriers and positive behavior when exposed to disorganized attachment **$\beta_{Met-carriers}$ = 0.85, *P* = 0.02** Met carriers exhibit more positive behavior than Val carriers at T1 **Met = Val/Val ($B_1 = B_2$) *P* = 0.009** Val carriers exhibit more negative behavior than Met carriers at T1 **Met = Val/Val (B1 = B2) *P* = 0.003**	No associations found from teacher reports of behavior – relationship specific externalized behavior
(vi) Viddal *et al.* 2017 TESS	5HTTLPR and attachment interact to predict emotion regulation Children with s/s allele and disorganized attachment at 4 to 6 years more likely to exhibit decreased emotion regulation from 6 to 8 years. Children with s/s allele and secure attachment at 4 to 6 years more likely to exhibit increased emotion regulation from 6 to 8 years.	Significant association between s/s allele and change in emotion regulation aged 6 to 8, dependent on change in attachment aged 4 to 6 **β = 0.63, *P* = .001**	

(vii) Borelli *et al.* 2017 California	FKBP5 and attachment interact to predict externalized behaviors relating to emotion regulation Child attachment security was inversely associated with RSA reactivity, emotional suppression, rumination, and depressive symptoms among children with high risk plasticity (ie. the CC allele) No significant associations found for children with AA or AC allele	CC allele x attachment security = inverse association with RSA reactivity **p=0.01** AA or AC allele x attachment security = no association with RSA reactivity CC allele x attachment security = inverse association with emotional suppression **p=0.004** AA or AC allele x attachment security = no association with emotional suppression CC allele x attachment security = inverse association with rumination **p=0.004** AA or AC allele x attachment security = no association with rumination CC allele x attachment security = significant inverse association with depressive symptoms **p=0.0001** AA or AC allele x attachment security = inverse association with depressive symptoms **p=0.03**	

Abbreviations: BEIP: Bucharest Early Intervention Project, RLS: Regensburg Longitudinal Study, SECCYD: Study of Early Child Care and Youth Development, TESS: Trondheim Early Secure Study,

Figure 1. PRISMA flowchart of identification and elimination of studies for review.

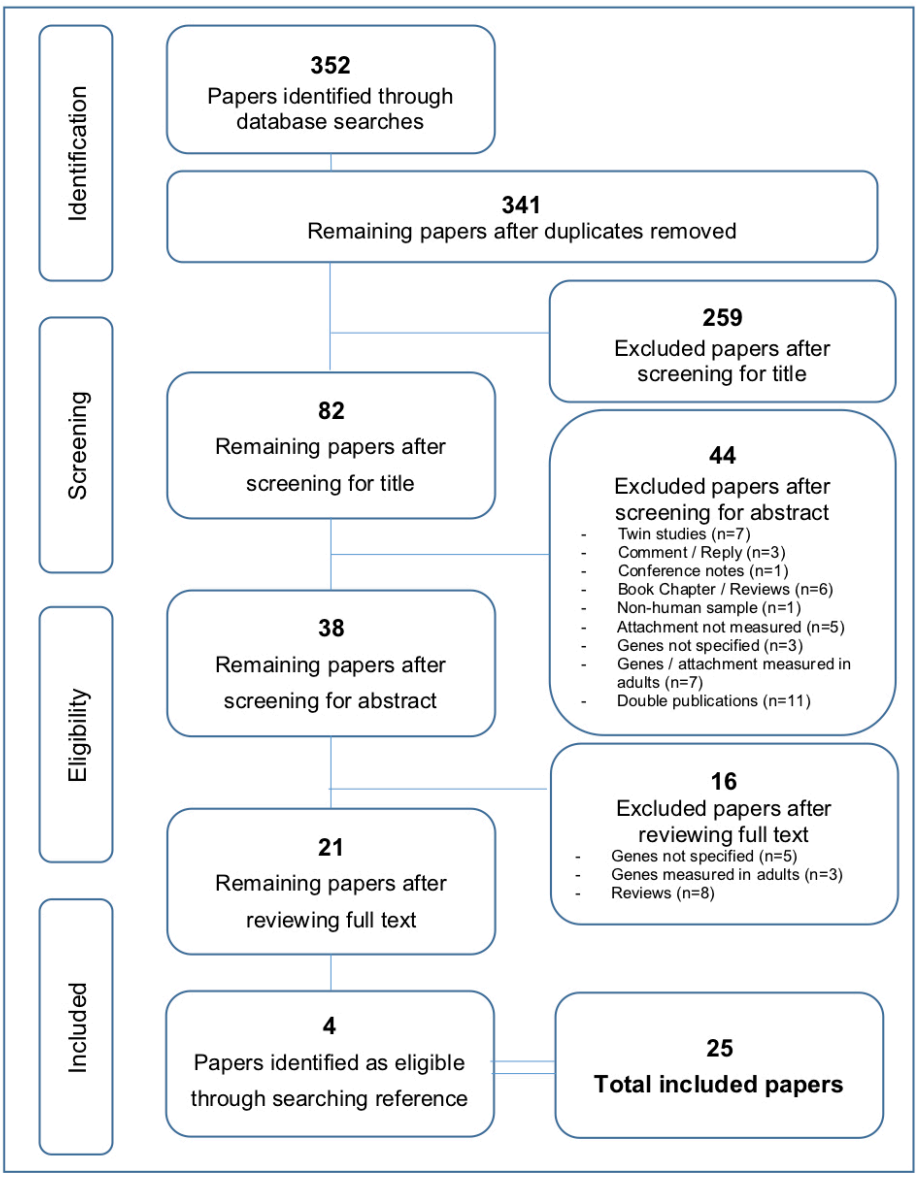

Figure 1. PRISMA flowchart of identification and elimination of studies for review

Figure 2: Suggested influences of Gene × Environment on attachment.

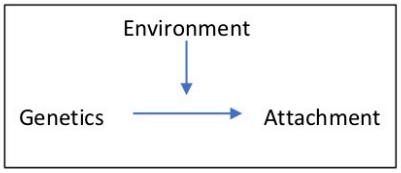

Fig 2. Suggested influences of Gene x Environment on attachment

Fig 3. Suggested influences of Gene x Environment (attachment) on behavior

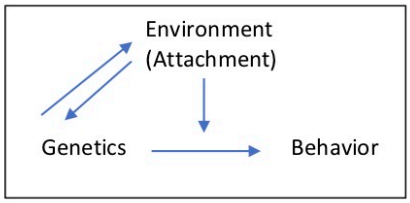

Fig 3. Suggested influences of Gene x Environment (attachment) on behavior